FLORAL PIGMENTATION PATTERNS IN *CLARKIA* (ONAGRACEAE)

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ABSTRACT

The pattern of anthocyanin pigmentation on flower petals of species of *Clarkia* sections *Rhodanthos* and *Godetia* is reviewed. The petals have large spots, blotches, or bands of red-purple color near the base, the center, the upper margin or in several positions, or are unspotted. A recent genetic analysis in *C. gracilis* revealed that the large central petal spot characteristic of subsp. *sonomensis* is allelic to a basal petal spot in subsp. *gracilis* that is normally not expressed because of the action of a modifier gene. Since *Clarkia* species display only a small number of discrete pigmentation patterns, I suggest that the major components of pattern differences among them are not complex from a genetic standpoint. Novel patterns can be assembled by substituting alleles at relatively few loci. Additional genes presumably contribute to various details of the patterns.

In many genera of plants, the flower displays more differences from species to species than any other plant part, varying, for example, in size, shape, outline, texture, orientation, and color as well as in features of scent, nectar, and pollen. Many floral traits serve to attract and reward specific pollinators. Although the flower has obvious and important adaptive significance, the genetic basis of floral differences between species has been worked out in only a few cases (Gottlieb 1984). Genetic studies might reveal whether new floral phenotypes result from the activities of a relatively small number of (major) genes or originate only after the accumulation of numerous genetic differences. In addition, when the same or similar phenotype appears in a number of species, it is important to determine whether this reflects the activities of the same subset of genes or convergence based on the independent appearance of new combinations of genes. Information on these issues will help us to understand how morphological differences evolve in plants.

Floral differentiation involving changes in both structural morphology and pigmentation patterns is particularly important among species of *Clarkia* (Onagraceae) native to California. Overall floral differentiation in *Clarkia* is closely associated with the pollination system (MacSwain et al. 1973). Indeed, much of the adaptive radiation in the genus has primarily involved the flower, so that if plants of different species were stripped of their flowers, they would be nearly identical in appearance (MacSwain et al. 1973). The genus has long been used as a model system for studies of plant evolu-

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tionary biology, beginning with the elegant biosystematic and cytogenetic studies of Professor Harlan Lewis and his students and colleagues (Lewis 1953, 1962, 1973; Lewis and Lewis 1955). More recent studies of *Clarkia* include examination of genetic differentiation among species using data from electrophoretic analysis of isozymes (Gottlieb and Weeden 1979; Pichersky and Gottlieb 1983; Odrzykoski and Gottlieb 1984; Soltis et al. 1987), and reconstructions of species relationships using restriction endonuclease analysis of chloroplast DNA (Sytsma and Gottlieb 1986a, b).

The most common floral type in diploid *Clarkia* species is the "godetia" flower which characterizes sections *Godetia, Rhodanthos, Peripetasma,* and *Fibula,* totalling about two dozen species. The godetia flower is held upright and is shaped like a bowl. The four petals are obovate to fan-shaped and are not much narrowed at the base. Although their structure, size, and shape are generally similar, the pattern of anthocyanin pigmentation, particularly on the petals, varies strikingly. The petals may have large spots, blotches, or bands of reddish-purple color near the base, the center, the upper margin, or sometimes in several of these positions. Alternatively, the petals may have hundreds of very small (2–8 cells) red or purple flecks, particularly near the center, or be unspotted. The characteristic lavender to reddish-purple pigments have been identified as glycoside derivatives of malvidin, supplemented with derivatives of cyanidin and delphinidin (Soltis 1986). The large spots or flecks appear to result from locally elevated levels of the same pigments, though in different proportion than in the petal background (Dorn and Bloom 1984). Although the petals are generally pigmented throughout, those of many species also have large white areas of no pigmentation. Anthocyanins may also be present or absent on filaments and anthers, the stigma, and the floral tube.

Petal Pattern in *Clarkia gracilis*

The petal pigmentation patterns of species assigned to *Godetia* and *Rhodanthos* can be divided into four major types: central spot only, distal spot only, basal band only, and unspotted. In addition, the petals of several species have more than one large pigmented spot. Since nearly all *Clarkia* species are strongly reproductively isolated, the genetic basis of the petal patterns cannot be directly studied. However, it has been possible to carry out a genetic study between two subspecies of *Clarkia gracilis,* an allotetraploid species in *Rhodanthos,* derived from the diploid *C. amoena* subsp. *huntiana* and an extinct species related to *C. lassenensis* and *C. arcuata* (Abdel-Hameed and Snow 1972).

Clarkia gracilis includes four interfertile subspecies that, as a group, display three of the four basic petal patterns described above. Sub-

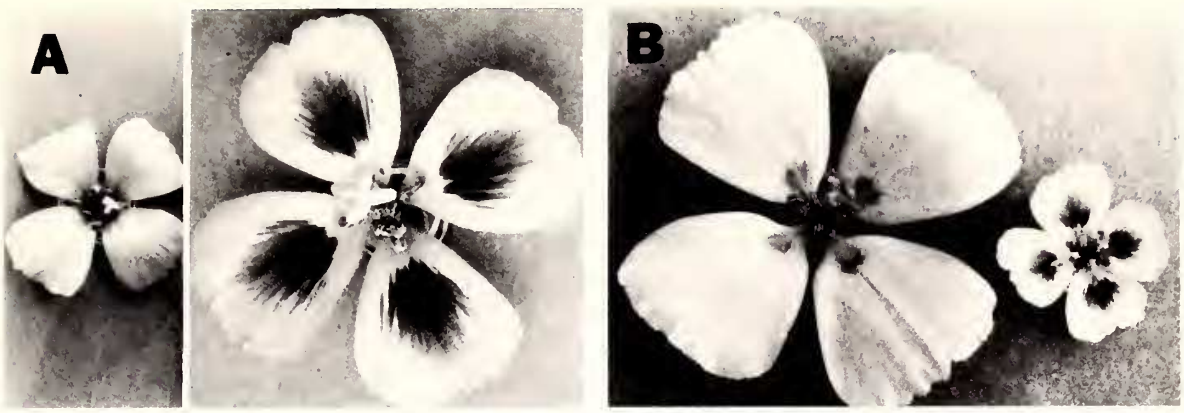
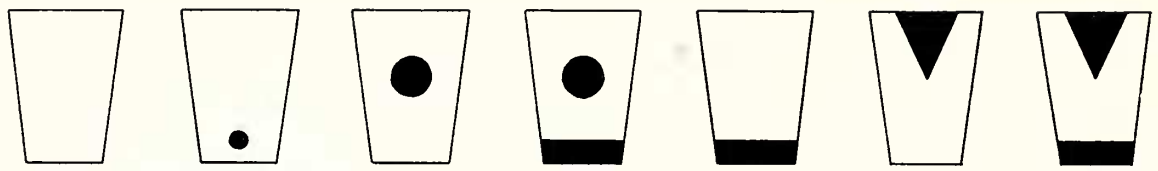


FIG. 1. (A) Flowers of *C. gracilis* subsp. *gracilis* (left), and *C. gracilis* subsp. *sonomensis* (right); (B) Flowers of F_2 recombinant with large flower and basal spot, and an F_2 recombinant with small flower and central spot.

species *sonomensis* has large pink petals each with a large central red-purple spot, subspp. *albicaulis* and *tracyi* have large pink petals each with an intense red-purple band of color across the base, and subsp. *gracilis* has small unspotted pink petals. The three subspecies with large petals have a marked protandry (the pollen is relased 3–5 days before the stigma becomes receptive) and are outcrossing. Subspecies *gracilis* is predominantly self-pollinating because when its flowers open, the stigma is already receptive and at the same height as the adjacent anthers. The four subspecies have a high genetic identity (Holsinger and Gottlieb 1988) and a moderate amount of chromosomal structure divergence (Abdel-Hameed and Snow 1968, 1972).

The petals of subsp. *sonomensis* are about 2.5 times longer and 4 times wider than those of subsp. *gracilis*. Numerous genes probably contribute to the size difference because the petal sizes of both parents were not recovered in a large F_2 (238 plants) or backcross progenies from hybrids between them (Gottlieb and Ford 1988). Although petal size behaves like a classical quantitative trait, the presence/absence of petal spots is controlled by a single gene. Thus a recent genetic analysis (Gottlieb and Ford 1988) revealed that subsp. *gracilis* has a gene governing basal petal spot that is not normally expressed (Fig. 1). The gene is allelic to one for central spot in subsp. *sonomensis,* but is not expressed because it is inhibited by a gene at another locus (Gottlieb and Ford 1988). Allelism of central petal spot, basal "band" (see below), as well as unspotted petal had previously been reported in the related diploid *C. rubicunda* (Rasmuson 1921; Hiorth 1940). Additional genes modify the size and exact position of the central spot. For example, the width of the central spot can vary from very narrow, with only a few dozen files of pigmented cells, to a broad blotch of color about half or more the width of the petal. The genetics of these modifications have not yet been analyzed.



GODETIA						
Nit		Nit			Imb	
Dav*		Pro*				
Pur*		Pur*		Pur*	Pur*	Pur*
Spe/i		Spe/p,s				
Ten*		Wil	Wil			
RHODANTHOS						
Arc				Arc		
Amo/l		Amo/a,c,h,w		Fra		
Gra/g*	(Gra/g*)	Gra/s*		Gra/a*,t*		
				Las		
				Rub		

FIG. 2. Diagram of petal pigmentation patterns in species of *Clarkia* sections *Godetia* and *Rhodanthos*. An asterisk indicates the species is polyploid. Names of the taxa are abbreviated as follows: *C. arcuata* (ARC); *C. amoena* subsp. *amoena* (AMO/a), subsp. *caurina* (AMO/c), subsp. *huntiana* (AMO/h), subsp. *lindleyi* (AMO/l), subsp. *whitneyi* (AMO/w); *C. davyi* (DAV); *C. franciscana* (FRA); *C. gracilis* subsp. *albicaulis* (GRA/a), subsp. *gracilis* (GRA/g), subsp. *sonomensis* (GRA/s), subsp. *tracyi* (GRA/t); *C. imbricata* (IMB); *C. lassenensis* (LAS); *C. nitens* (NIT); *C. prostrata* (PRO); *C. purpurea* (PUR); *C. rubicunda* (RUB); *C. speciosa* subsp. *immaculata* (SPE/i), subsp. *polyantha* (SPE/p), subsp. *speciosa* (SPE/s); *C. tenella* (TEN); *C. williamsonii* (WIL).

PETAL PATTERN IN RELATED *CLARKIA* SPECIES

Knowledge of the genetic basis of petal pigmentation in *C. gracilis* suggests that many of the petal patterns that distinguish other species in *Godetia* and *Rhodanthos* may also be governed by single genes. The petal patterns of all the species in the two sections are diagrammed in Figure 2. Most species are spotless, or have a central spot or a basal band of color. Two species have a wedge-shaped spot that extends from the center of the petal to its upper margin. Four species (*C. arcuata, C. nitens, C. purpurea,* and *C. williamsonii*) are polymorphic for petal pattern. Of them, the most polymorphic is *C. purpurea,* an allohexaploid that has at least five common patterns, all frequently observed in the same population.

The many variants of *C. purpurea* probably reflect its hexaploidy and the consequences of occasional hybridization followed by sorting out of new homozygous genotypes by virtue of its self-pollinating breeding system. Lewis and Lewis (1955, p. 304) reported a cross between the small-flowered *C. purpurea* subsp. *quadrivulnera* and the large-flowered subsp. *viminea* that showed continuous segregation for petal size in an F_2 but sharp segregation for petal color and pattern, similar to results obtained in *C. gracilis* (Gottlieb and Ford

1988). Apparently homologous genes affecting floral pigmentation in diploid and tetraploid derivatives have been described in *Primula* (De Winton and Haldane 1933) and *Gossypium* (Harland 1935).

Clarkia williamsonii often exhibits both central spot and basal band on its petals. Since the two are known to be allelic in another *Clarkia* (Rasmuson 1921; Hiorth 1940), their combined presence in a true-breeding pattern in a diploid species is consistent with duplication of the coding genes, analogous to duplication of genes encoding isozymes described for a number of diploid *Clarkia* species (Gottlieb 1977; Pichersky and Gottlieb 1983). Alternatively, the multiple spots may reflect activation of a single gene at different times during petal development.

Since clarkias display only a small number of discrete petal patterns, and since differences in several of them are allelic, it is plausible that the presence of the same petal pattern reflects utilization of the same subset of genes, and that different patterns result from simple allelic substitutions. Many additional genes presumably contribute to the details of floral pigmentation, for example, the particular pigment mix, the size and shape of the spots (round versus wedge-shaped) and their exact position on the petal. The inheritance of the major components of floral pattern, for example, spot presence/absence and spot position, however, need not be regarded as complex. New patterns can be assembled by substituting alleles at a small number of loci, not unlike the situation described for cuticular patterns of chaetae and trichomes in *Drosophila* (Garcia-Bellido 1983).

The Basal Spot Gene in subsp. *gracilis*

The recovery of an allele for basal spot from the unspotted subsp. *gracilis* poses interesting questions about its origin. The basal band of color in the related subspecies, *albicaulis* and *tracyi,* extends completely across the petal base whereas the subsp. *gracilis* spot is small, more or less round, and does not extend to the edges of the petal. The gene for large central spot in subsp. *sonomensis* most likely comes from *C. amoena* subsp. *huntiana* which also has a similar central petal spot, and the gene for basal band characteristic of subspp. *albicaulis* and *tracyi* probably derives from a species related to the diploids *C. lassenensis* and *C. arcuata* which have a basal petal band. The two other diploid species in *Rhodanthos* also have a basal petal band.

Natural hybridization between subsp. *gracilis* and subsp. *sonomensis* has been documented (Lewis and Lewis 1955, p. 280), and it is interesting that plants with small petals and central spots that otherwise resemble subsp. *gracilis* were identified. Were some plants to show the novel basal spot, an observer would not know that its

coding gene already existed, and was simply "released" from inhibition. Many loci may include alleles that normally remain unexpressed, and segregation following hybridization, which is frequent in plants, may place such alleles as well as normally expressed alleles under new patterns of regulation resulting in the abrupt appearance of new forms. Though they might seem like macromutations, the novel phenotypes could be simply explained. Novel phenotypes would also occur when a rare recessive allele that governs a morphological trait in an outcrosser becomes homozygous and is expressed following hybridization with a related selfer (Rick and Smith 1953).

The progenies between subsp. *gracilis* and subsp. *sonomensis* also segregated for several other differences in their pigmentation patterns (Gottlieb and Ford 1988). The background petal color of subsp. *sonomensis* is frequently uniform. However, in some individuals, the basal quarter of each petal lacks anthocyanin pigments and is bright white, giving the appearance of a "white cup," especially when the flower is newly opened. Presence versus absence of white cup proved to be governed by a single locus, with white cup (representing absence of pigment) recessive. A single gene was also identified that governs presence versus absence of dark red pigmentation on the inner surface of the floral tube, and another gene was found that controls presence versus absence of color on the anthers and filaments. In all, five genes that affect pattern of anthocyanin color on the petals and other floral organs were identified in addition to the polygenic control of petal size. The white cup pattern is present in many *Clarkia* species including *C. arcuata, C. bottae, C. davyi,* and *C. imbricata* as well as in *C. gracilis* subspp. *albicaulis* and *tracyi,* and its genetic basis may be similar to that found in subsp. *sonomensis.* Presence or absence of color on the floral tube also distinguishes *Clarkia* species.

Conclusions

It is not known whether the differences in pigmentation pattern of *Clarkia* species were selected by different pollinators. MacSwain et al. (1973) believed that the differences in petal spotting may play a role in determining pollinator constancy on individual trips from the nest, thus serving to increase the frequency of intraspecific pollinations. Some of the novel variants of *C. gracilis* constructed during the genetic study may be useful to test hypotheses concerning floral pattern and pollination system. Appropriate variations can be introduced into different habitats and monitored to observe pollinator preferences. In such studies, it would be possible to focus on individual traits such as spot position or white cup or on different trait combinations.

The discovery that subsp. *gracilis* has a gene for basal petal spot that is not normally expressed because of the action of a second gene emphasizes the importance of genetic studies for understanding morphological differences between species. This may be particularly significant in plants where interspecific hybridization and allopolyploidy are prevalent. Many novel phenotypes in plants may result from new modes of gene regulation brought about by the juxtaposition of divergent genetic materials rather than by accumulation of novel genes.

The pigmentation pattern on petals and other floral organs commonly varies among species in many plant genera. Important genetic studies in cultivated plants include the analysis of Japanese morning glories which revealed a large number of single genes that confer sharply distinct pigment patterns on the petals such as *blizzard, flecked, lined, striated, speckled,* and *smeary* (Imai 1931). Other genetic studies were carried out in *Primula sinensis* (De Winton and Haldane 1933). Few studies have been done in wild plants although many reports are available of pattern differences between and within species. A recently published example is *Platystemon californicus,* which exhibits at least six color patterns on its showy flowers that appear to be roughly correlated with geographical distribution (Hannan 1981). A number of species with flower color polymorphism are listed in Hannan (1981) and Kay (1978).

The flower is a complex structure in which many specialized tissues and cell types form distinctive organs in a precise and orderly manner. The differentiation of structures is most likely independent of pigmentation pattern, and this is one reason the patterns may be changed by few genes. Although the patterns may have a simple and readily modified developmental basis, pigment patterns are likely to have complex effects on pollination and eventual seed set. Changed patterns appear to be particularly important in annual plants like *Clarkia* in which most species are outcrossing, and often as many as five or six species grow intermixed beneath the oak trees on the same Sierran hillside.

Literature Cited

Abdel-Hameed, F. and R. Snow. 1968. Cytogenetic studies in *Clarkia,* sect. *primigenia.* IV. A cytological survey of *Clarkia gracilis.* Amer. J. Bot. 55:1047–1054.

——— and ———. 1972. The origin of the allotetraploid *Clarkia gracilis.* Evolution 26:74–93.

De Winton, D. and J. B. S. Haldane. 1933. The genetics of *Primula sinensis.* II. Segregation and interaction of factors in the diploid. J. Genetics 27:1–44.

Dorn, P. S. and W. L. Bloom. 1984. Anthocyanin variation in an introgressive complex in *Clarkia.* Biochem. Syst. Ecol. 12:311–314.

Garcia-Bellido, A. 1983. Comparative anatomy of cuticular patterns in the genus *Drosophila.* Pp. 227–259 *in* B. C. Goodwin, N. Holder, and C. C. Wylie (eds.), Development and evolution. Cambridge Univ. Press, Cambridge.

GOTTLIEB, L. D. 1977. Evidence for duplication and divergence of the structural gene for phosphoglucose isomerase in diploid species of *Clarkia*. Genetics 86: 289–307.

———. 1984. Genetics and morphological evolution in plants. Amer. Naturalist 123:681–709.

——— and V. S. FORD. 1988. Genetic studies of the pattern of floral pigmentation in *Clarkia gracilis*. Heredity 60:237–246.

——— and N. F. WEEDEN. 1979. Gene duplication and phylogeny in *Clarkia*. Evolution 33:1024–1039.

HANNAN, G. L. 1981. Flower color polymorphism and pollination biology of *Platystemon californicus* Benth. (Papaveraceae). Amer. J. Bot. 68:233–243.

HARLAND, S. C. 1935. Homologous genes for anthocyanin pigmentation in new and old world cottons. J. Genetics 30:465–476.

HIORTH, G. 1940. Eine Serie multipler Allele für Blütenzeichnungen bei *Godetia amoena*. Hereditas 26:441–453.

HOLSINGER, K. and L. D. GOTTLIEB. 1988. Isozyme variability in the tetraploid *Clarkia gracilis* (Onagraceae) and its diploid relatives. Syst. Bot. 13:1–6.

IMAI, Y. 1931. Analysis of flower color in *Pharbitis nil*. J. Genetics 24:203–224.

KAY, Q. O. N. 1978. The role of preferential and assortive pollination in the maintenance of flower color polymorphisms. Pp. 175–190 *in* A. J. Richards (ed.), The pollination of flowers by insects. Academic Press, London.

LEWIS, H. 1953. The mechanism of evolution in the genus *Clarkia*. Evolution 7: 1–20.

———. 1962. Catastrophic selection as a factor in speciation. Evolution 16:257–271.

———. 1973. The origin of diploid neospecies in *Clarkia*. Amer. Naturalist 107: 161–170.

——— and M. E. LEWIS. 1955. The genus *Clarkia*. Univ. Calif. Publ. Bot. 20:241–392.

MACSWAIN, J. W., P. H. RAVEN, and R. W. THORP. 1973. Comparative behavior of bees and Onagraceae. IV. *Clarkia* bees of the western United States. Univ. Calif. Publ. Entom. 70:1–80.

ODRZYKOSKI, I. J. and L. D. GOTTLIEB. 1984. Duplications of genes coding 6-phosphogluconate dehydrogenase in *Clarkia* (Onagraceae) and their phylogenetic implications. Syst. Bot. 9:479–489.

PICHERSKY, E. and L. D. GOTTLIEB. 1983. Evidence for duplication of the structural genes coding plastid and cytosolic isozymes of triose phosphate isomerase in diploid species of *Clarkia*. Genetics 105:421–436.

RASMUSON, H. 1921. Beiträge zu einer genetischen Analyse zweier *Godetia*-Arten und ihrer Bastarde. Hereditas 2:143–289.

RICK, C. M. and P. G. SMITH. 1953. Novel variation in tomato species hybrids. Amer. Naturalist 87:359–373.

SOLTIS, P. S. 1986. Anthocyanin variation in *Clarkia* (Onagraceae). Biochem. Syst. and Ecol. 14:487–489.

———, D. E. SOLTIS, and L. D. GOTTLIEB. 1987. Phosphoglucomutase gene duplications in *Clarkia* (Onagraceae) and their phylogenetic implications. Evolution 41:667–671.

SYTSMA, K. J. and L. D. GOTTLIEB. 1986a. Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (Onagraceae). Evolution 40: 1248–1261.

——— and ———. 1986b. Chloroplast DNA evidence for the origin of the genus *Heterogaura* from a species of *Clarkia* (Onagraceae). Proc. Nat. Acad. Sci. U.S.A. 83:5554–5557.

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